CHOLINOCEPTIVE AND ADRENOCEPTIVE PROPERTIES OF THE TUNICATE HEART PACEMAKER

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Functionally, tunicate hearts resemble those of the higher chordates since conduction is from cell to cell by local current flow (Kriebel, 1967b, 1968c, 1968d) and the pacemakers are myogenic (Millar, 1953; Kriebel, 1968b). However, cardiovascular control in tunicates has not been extensively studied.

There are no continuously active nerves that maintain the beat frequency either above or below the intrinsic frequency of the pacemaker (something like vagal tone in the higher vertebrates) since ganglion extirpation does not alter the beat frequency (Day, 1921; Bacq, 1935). Stimulation of the ganglion is without effect on the heart rate (Schultze, 1901). However, it is not excluded that there are cardiovascular nerves that are active only during specific conditions.

If regulatory nerves are present, it seems likely that the transmitters would be the same as in the higher chordate. Although acetylcholine and cholinesterase are present in tunicates (Florey, 1963), Ach at low concentrations has no effect on the beat frequency of intact isolated hearts (see Krijgsman, 1956, for a review; Krijgsman and Krijgsman, 1959). Adrenaline has been reported to accelerate the heart beat, but high concentrations were required (10^{-3} g./ml.; Scudder *et al.*, 1963).

Since there are no transverse channels (or gaps) between cells, the myocardium is an effective barrier to substances that do not penetrate cell membranes (Kriebel, 1968d). The results presented here show that Ach and adrenaline do not cross the heart wall and that receptive sites are present only on the lumen surface.

Methods

Hearts of large adult *Ciona intestinalis* (from California) were exposed by cutting through the test and body wall into the coelomic cavity (see Kriebel, 1968a). Hearts ranged from 24 to 45 mm. in length. In some preparations the blood vessels were ligated before cutting in order to free the heart, whereas in others, the vessels were simply severed. In all preparations the pericardium was opened. Many isolated hearts were ligated in the middle region so that the activity of one primary pacemaker could be studied without the influence of the opposite pacemaker. Hearts were opened so that the lumen surface was exposed to the bath either by cutting along the raphe, or, during electrical recording, by pressing a razor blade through the myocardium. The latter method of opening hearts did not disturb the tissue that was sucked into suction electrodes (Kriebel, 1968a).

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Hearts were carefully secured with small hooks to the bottom of a 5-ml. bath (10° C. or 21° C.) to insure that constant dimensions in the heart wall were maintained during addition of the drug solutions and the washing procedure. Slight alterations in tension changed the amount of tissue sucked into the electrode openings and thereby altered the signal amplitude as well as the excitability of the pacemakers (Kriebel, 1968a). Consequently, control experiments, which consisted of adding, mixing and removing sea water, were performed before and after the drug additions to validate the drug actions. Hearts with pacemakers that initially or later became sensitive to alterations in the bath volume were not used.

The effect of drugs on the beat frequency was tested on hearts, first intact, then opened. A solution of the drug in sea water at a temperature equal to that of the bath was added with a pipette. The final concentrations were predetermined. Mixing was accomplished within 15 seconds with an eye dropper. To remove a drug solution at least 100 ml. of sea water of the desired temperature were perfused through the bath.

Acetylcholine chloride (Merck), L epinephrine bitartrate (Nutritional Biochemical Corporation), d-tubocurarine chloride pentahydrate (Burroughs Wellcome & Co.) and atropine sulphate (The Norwich Pharmacal Co.) were used.

Results

Since the effects of drugs on the pacemaker frequencies of intact and opened hearts were to be compared, and since the amount of intracardiac blood affects the beat frequency (Kriebel, 1968a), intact hearts were studied in the collapsed condition (*i.e.*, they contained no blood). Thus, when the hearts were opened, the pacemakers were not subjected to a change in tension. However, arrhythmia frequently occurred in collapsed hearts (Kriebel, 1968a, 1968b). A common pattern of arrhythmia was a doubling in the beat frequency due to the activity of an ectopic center (as shown in Fig. 1A, trace 1).

Acetylcholine (10^{-8} to 10^{-4} g./ml.) and adrenaline (10^{-6} to 5×10^{-4} g./ml.) were without effect on the beat frequency of intact isolated *Ciona* hearts (and half-hearts; with and without the blood vessels tied off, full of blood or collapsed). However, when the hearts were opened, forming a flat sheet of tissue, Ach at 10^{-8} to 10^{-7} g./ml. or adrenaline at 10^{-5} g./ml. initially stopped the primary pacemaker (Figs. 1 and 2). The pacemakers did not again stop when the bath was stirred or when another threshold aliquot of drug was added. The concentration could be slowly raised to about 100 times threshold for Ach and about 10 times for adrenaline with no effect on the beat frequency of the primary pacemaker. Therefore, the response of the pacemakers to these agents exhibited tachyphylaxis, *i.e.*, they became desensitized. However, at initial concentrations greater than 100 × threshold for Ach and 10 × threshold for adrenaline the primary pacemakers were irregular and ectopic centers developed (Ach at 10^{-5} g./ml., Fig. 2; and adrenaline at 5×10^{-4} g./ml., Fig. 1).

Even when vessels were not ligated; intact isolated hearts were insensitive to the drugs, presumably because the blood vessels sealed when they were severed. When the vessels were opened and washed, intact hearts became sensitive to Ach and adrenaline. However, the concentrations required to arrest the hearts were

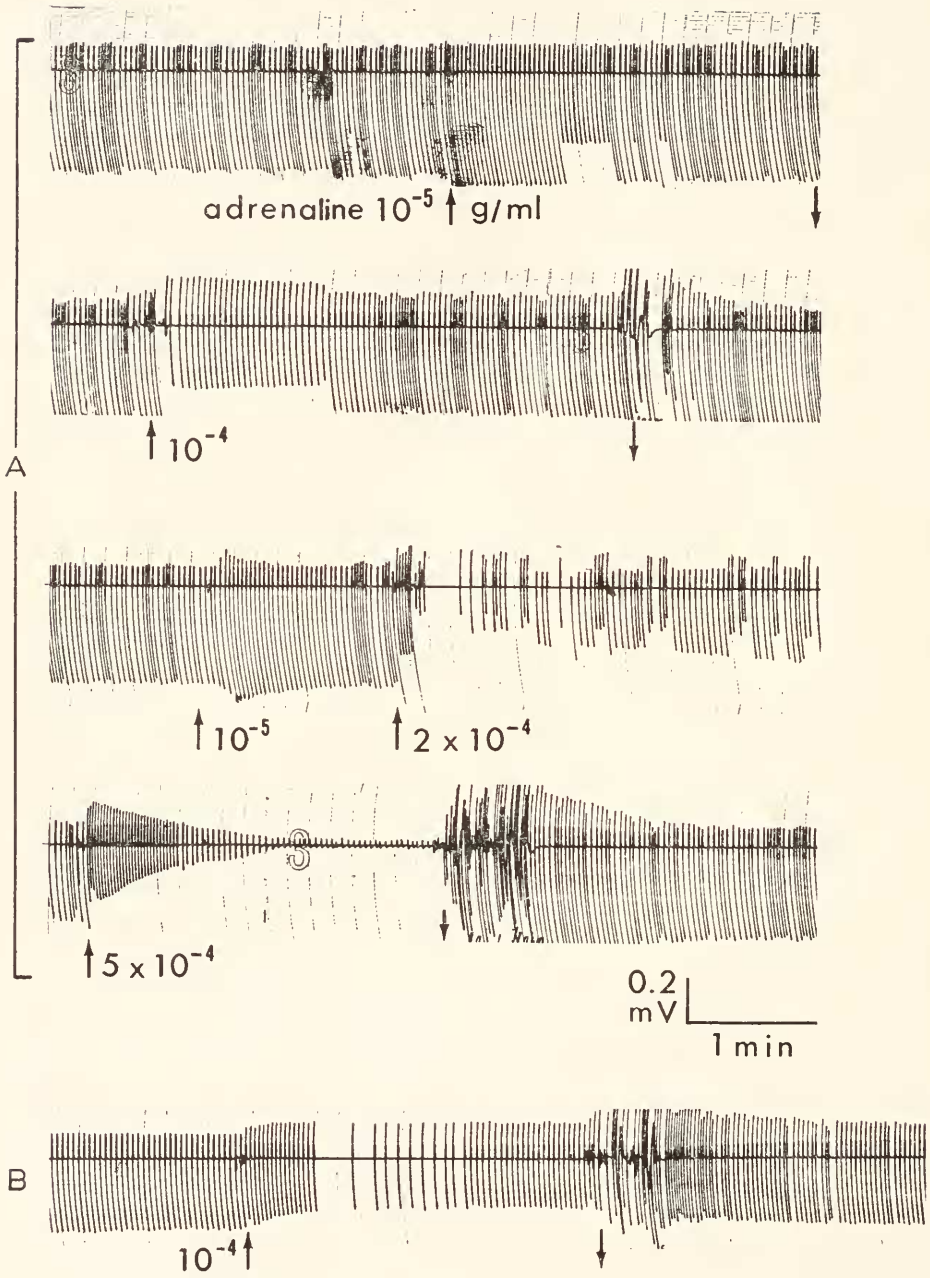
adrenaline 10^{-5} g/ml
10^{-4}
A
10^{-5}
2×10^{-4}
5×10^{-4}
0.2 mV
1 min
B
10^{-4}

FIGURE 1.

higher than required after the hearts were opened. This indicates that diffusion through the collapsed ostia is slow and in addition it is likely that tachyphylaxis occurred.

Atropine antagonized the effect of Ach (Fig. 2). Threshold concentrations of d-tubocurarine (10^{-7} to 3×10^{-7} g./ml.) either slowed or stopped a pacemaker center. Complete stoppage of the heart occurred with slightly greater concentrations and primary pacemakers did not recover. The effect was additive to that of Ach.

Adrenaline sometimes stopped the heart for a few beats permitting an ectopic center to develop which alternated with the primary pacemaker center, doubling the beat frequency (Fig. 1A, trace 4; *cf.* Scudder et al., 1963; Sugi and Matsunami, 1966). Doubled beat frequency also occurs frequently in deflated hearts and can be produced by locally heating the pacemaker region (Kriebel, 1968a, 1968b). If a primary pacemaker and an ectopic center were active in a heart arm, a threshold concentration of adrenaline (10^{-5} g./ml.) often stopped one center, resulting in halved beat frequency (Fig. 1A, trace 1).

High concentrations of adrenaline (5×10^{-4} g./ml.) decreased conduction velocity (Kriebel, 1967a). This can explain the decrease in signal amplitude (also found by Scudder *et al.*, 1963). After addition of a high concentration of adrenaline, conduction velocity continued to decrease for 1 to 3 minutes. During this time, amplitude of action potentials also gradually decreased (Fig. 1A, trace 4). Although beat frequency decreased under the influence of adrenaline, groups of beats at a relatively high frequency occurred. By the shape of the recorded wave form they could be attributed to one pacemaker and it appears that beats were missed between the groups (as seen in Fig. 1A, trace 3). Because conduction velocity is very low and because it is decremental at the ends of the heart (Kriebel, 1967a) a further decrease in the conduction velocity due to the action of adrenaline would decrease the safety margin for conduction. A reduced safety margin could give rise to missed beats and permit the development of ectopic centers.

FIGURE 1. Effect of adrenaline on the beat frequency of opened *Ciona* hearts. The electrograms were recorded with suction electrodes placed on the primary pacemaker region. The downward arrows indicate washing; 20° C. A. Trace 1: The first half of this record shows a rhythmical doubling in beat frequency attributed to the activity of an ectopic center which alternated with the activity of the primary pacemaker. After the application of adrenaline note that the doubling in beat frequency was stopped for over 1 minute. Trace 2: After the application of adrenaline at a concentration of 10^{-4} g./ml. the heart stopped, the doubling in frequency was abolished and for about 2 minutes the heart contracted at a lower frequency. However, it was visually determined that the direction of contraction had reversed. That is, an ectopic center at the cut end of the heart arm now drove the heart. The activity of the ectopic center is indicated by the change in the signal amplitude. Trace 3: With adrenaline 10^{-5} g./ml. the doubling in beat frequency was stopped as in trace 1. With adrenaline 2×10^{-4} g./ml. the heart did not recover. Signals of different amplitude indicate a change in the direction of conduction and thus the activity of different centers. Note that the signals of the same amplitude sometimes occurred in groups (see text). Trace 4: Continuous with trace 3. Adrenaline at 5×10^{-4} g./ml. doubled the beat frequency for about 40 seconds. Then the beat frequency suddenly dropped to about half, at which time it was visually observed that the wave of contraction started at the cut end of the half-heart. B. This electrogram shows the typical effect of adrenaline on an active pacemaker. The slight increase in frequency just after drug application was probably due to an increase in the bath temperature. Mixing was 30 sec. after arrow.

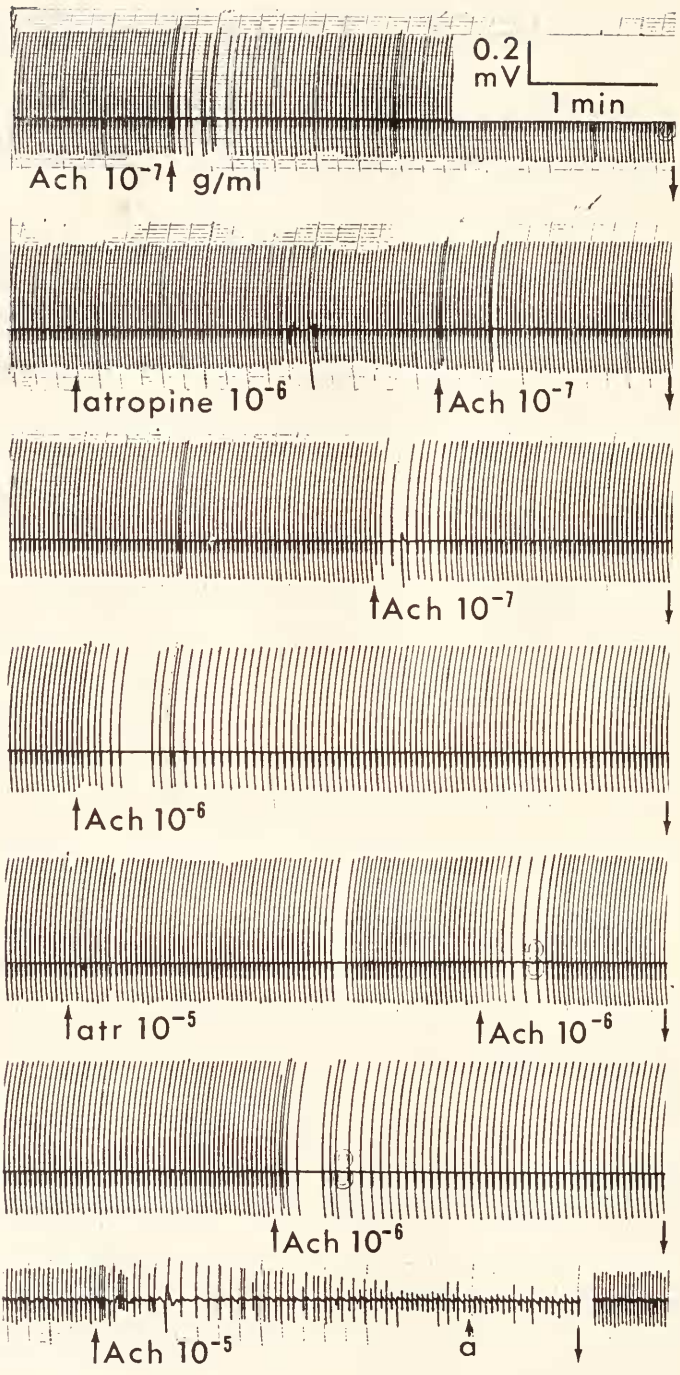
0.2 mV
1 min
Ach 10⁻⁷ g/ml
atropine 10⁻⁶
Ach 10⁻⁷
Ach 10⁻⁷
Ach 10⁻⁶
atr 10⁻⁵
Ach 10⁻⁶
Ach 10⁻⁶
Ach 10⁻⁵
a

FIGURE 2.

Discussion

Tight junctions join all cells together at their apical borders and exclude extracellular space so that there are no transepithelial diffusion channels between cells (Kriebel, 1968d). The ineffectiveness of drugs on intact hearts shows that the heart tube is permselective and that cholinoceptive and adrenoceptive sites are on the lumen surface.

The failures of other investigators to observe the effects of low concentrations of Ach and adrenaline are readily explained by the insensitivity of the unopened heart, the sealing of the cut ends of the heart and by the presence of tachyphylaxis (*cf.* Bacq, 1934; Krijgsman and Krijgsman, 1959; Scudder *et al.*, 1963; Sugi and Matsunami, 1966).

The absence of an accelerating effect of adrenaline, as found in other chordate hearts, may be an indication of the absence of cardioacceleratory nerves.

On the other hand, the typical cholinoceptive properties of the myocardium (inhibition caused by Ach and Ach-block by atropine) favors the interpretation that the tunicate heart may receive cardioinhibitory innervation (Florey, 1951) and this innervation is cholinergic. In this connection it is interesting that Bone and Whitear (1958) could trace nerves as far as the pericardium.

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Summary

1. Many previous investigators have reported that acetylcholine has little or no effect on the beat frequency of intact but isolated hearts. However, it was found that when hearts were split open, Ach at low concentrations (10^{-8} g./ml.) stopped the heart beat for up to a minute. Atropine blocked Ach.

2. D-tubocurarine at a concentration of 3×10^{-7} g./ml. stopped the beat of opened hearts. Its effect was additive to that of Ach.

3. Adrenaline (10^{-5} g./ml.) could stop pacemakers. However, a doubling in beat frequency was frequently observed with higher concentrations. Doubling in frequency resulted from an ectopic center which alternated with the primary center, thus driving the heart at twice its original frequency. In hearts which showed arrhythmia resulting from activity of more than one pacemaker center,

Figure 2. Effect of acetylcholine on the beat frequency of an opened *Ciona* heart. The electrograms were recorded with a suction electrode placed on the myocardium near the end of the heart (*i.e.*, on the primary pacemaker region). The downward arrows indicate washing. Temperature 20° C. Traces 1–3 show that atropine blocked a threshold concentration of Ach. Traces 4–6 show that atropine partially blocked the initial stoppage caused by a high concentration of Ach (10^{-6} g./ml.) and greatly diminished the time required to regain the original beat frequency. Trace 7 is from the same heart but the electrode position had been slightly changed altering the signal amplitude. Note the different amplitude after application of Ach. It was visually observed that the smallest signals (a) corresponded to contractions starting at the cut end of the half-heart.

adrenaline at a low concentration (10^{-5} g./ml.) usually stopped one or the other of the centers, decreasing the beat frequency by half.

4. Hearts recovered in low concentrations of either Ach or adrenaline. However, at higher concentrations (Ach 10^{-6}, adrenaline 5×10^{-4} g./ml.) arrhythmia was induced and hearts did not recover. The occurrence of arrhythmia is discussed with respect to the decremental nature of conduction in the ends of the heart and to the observation that all cells near the ostia are latent or dormant pacemakers.

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